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Myung Sook Kim, In Kyu Lee and Sung Min Boo: Phenology and morphology on *Gracilaria verrucosa* (Rhodophyta) on the west coast of Korea: A statistical approach

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Gracilaria verrucosa (Hudson) Papenfuss is well known as one of the most intensively studied red algal taxa, but the species concept is very problematic in that relatively few clear-cut morphological and anatomical criteria are available to distinguish the species (Bird and McLachlan 1982). Morphologically similar plants from different regions have been reported under different names (see Fredericq and Hommersand 1989), whereas Rice and Bird (1990) concluded, after comparing organelle DNA profiles between local and regional populations, that Japanese and Argentine plants as well as European ones under the name *G. verrucosa* were sufficiently similar to be considered conspecific. This conclusion still raises questions about the anatomical differences found between European and northwest Pacific plants (Zhang and Xia 1985). Japanese *G. verrucosa* plants, furthermore, have been known to be interfertile with *G. vermiculophylla* (Ohmi) Papenfuss (Yamamoto and Sasaki 1988).

There have been reports on northwest Pacific *G. verrucosa* in culture as well as in the field (see Yamamoto 1984, Yamamoto and Sasaki 1987). Although the structure and reproduction has been intensively studied by Yamamoto (1978), there is little information on temporal variation of morphology in the field. This study was carried out to elucidate the phenology and morphological variation in the field throughout the year.

The study site is located at the western side of the Daechon harbour (36° 19' N, 126° 30' E) on the west coast of Korea. The site is subjected to moderate wave activity and diurnal tidal fluctuation with a maximum height of 6 m. A large bed of *Gracilaria verrucosa* plants covered littoral and sublittoral fringe. Sampling was carried out in the flat littoral area of sand at monthly intervals from July 1988 to June 1989.

Fifty or more plants were collected at random and preserved in 5-10% formalin-seawater for phenology. Plants collected were observed for reproductive organs and sections were made when necessary. Twenty-five plants were randomly selected from each collection and the following vegetative features were measured: (1) total plant length including branch lengths; (2) axis diameter; (3) central medullary cell size in the middle portion of main axis (five slices); (4) basal branch constriction calculated by measuring branch diameter (a) at the basal portion of branches and (b) at the portion 10 mm distal from the base (five first-order branches from the middle part of each plant) (Fig. 1B, Chapman *et al.* 1977). Voucher specimens of *G. verrucosa* used in the pesent study were deposited in the herbarium of the Department of Biology at the Seoul National University.

The results were analysed using descriptive statistics (mean and standard deviation), multivariate analysis of variance (ANOVA-two way), student's T-test and Pearson's moment product correlation coefficient. Tests were conducted with the SAS computer program (Statistical Package) and only significant differences ($p<0.001$) are discussed in the text. Morphological data were also standardized by Doyle's formula (Radford 1986); $Z=(X-\bar{X})/S$, where Z represents the stan-

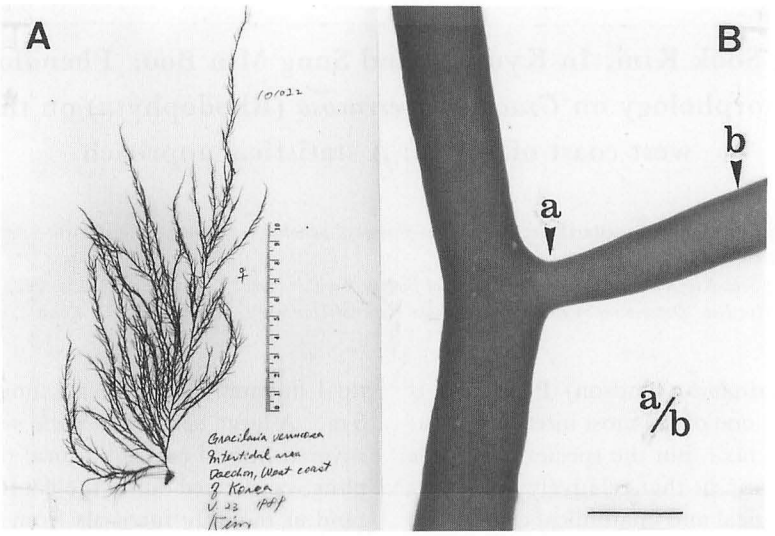

Fig. 1. *Gracilaria verrucosa* plant from the west coast of Korea: (A) a mature female with cystocarps collected in May, 1989 and (B) basal portion of branch measured for branch constriction (Scale bar 0.3 mm).

dardized variable, X the individual value, $\overline{X}$ the mean, and S the standard deviation.

Life history phases: A total of 750 plants were examined for phenology. Korean *Gracilaria verrucosa* plants (Fig. 1A) showed a *Polysiphonia*-type life history with a marked reproductive seasonality: reproductive plants (both tetrasporic and sexual plants) dominated from May to September, while vegetative thalli were abundant from November to April (Fig. 2). The total number of tetrasporic and sexual plants (female plus male plants) was also represented by similar proportions, although there were differences in the timing of their monthly peaks. Tetrasporic plants showed a high occurrence from May to October when vegetative ones were rarely seen, whereas from November to April the reverse was observed. Male plants occurred at a frequency below 30% of the total sample throughout the year. The same is true for female plants with the exception during June and July. Male, female, tetrasporic and vegetative plants observed in the present study were 151, 148, 271 and 180 individuals, respectively, thus giving the mean proportion of 20 : 20 : 36 : 24 throughout the year.

Reproduction and size class: A total of 510 individuals were examined to elucidate the relationship between plant length and reproductive states. Plants in different life history phases showed different peaks in size class (Fig. 3). Reproductive organs were observed on plants beginning at a size class of 4–8 cm. Male plants had a mean length of 11.8 ± 3.9 cm and showed a peak within a 8–12 cm range, while female ones had a mean length of 13.8 ± 9.0 cm and showed a peak in a 4–12 cm range.

Tetrasporic plants showed the largest mean (16.2 ± 10.7 cm) and the maximum length (60 cm), but most tetrasporic plants occurred in a size class of 8–12 cm. Vegetative plants belonged mostly within a 12–16 cm range. All the plants over 36 cm were reproductive, but few male and vegetative plants ranged over 24 cm.

Morphological variation: Plants collected were a maximum of 57 cm and a minimum of 3 cm, averaging 13.5 ± 7.7 cm ($n = 510$). Monthly mean of plant length was about 10 cm from July to November and then significantly increased to 28 cm in next June ($F = 94.58$, $p < 0.001$). The minimal length was 7.3 ± 1.9 cm in October 1988 and the maximum 28.5 ± 8.2 cm in June 1989 (Fig. 4), that was also significantly different ($F = 11.21$, $p < 0.001$) in different life history

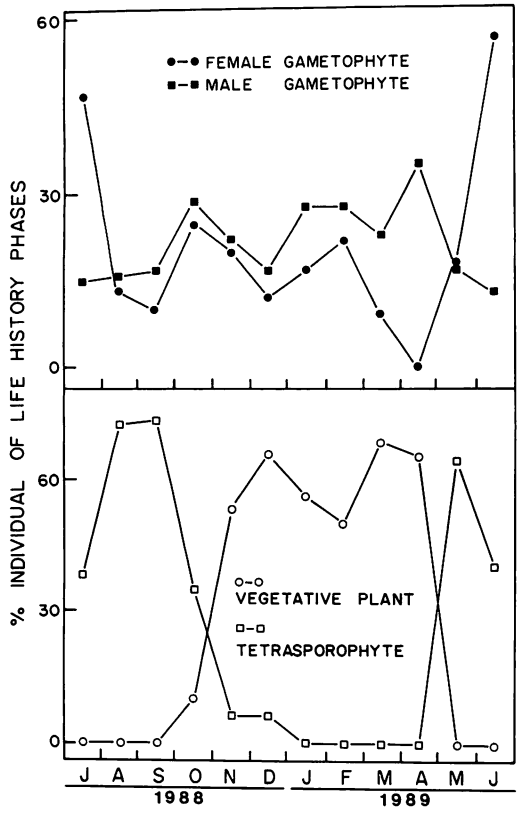

Fig. 2. Monthly variations in the reproductive states of individual Korean *Gracilaria verrucosa* plants.

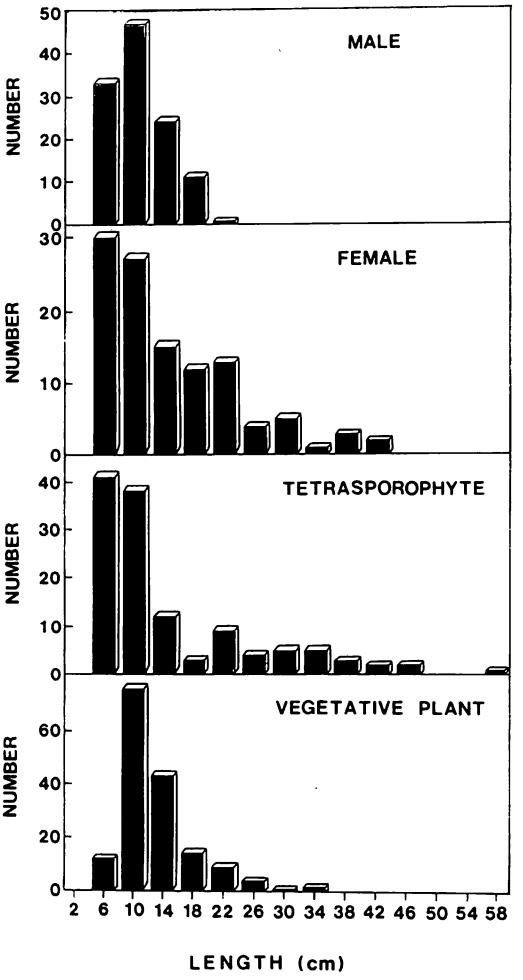

Fig. 3. Relationship between length and reproductive states in Korean *Gracilaria verrucosa* plants. Plotted are total number of individuals examined per 4 cm size class.

phases.

Basal branch constriction (Fig. 1B) varied significantly in different months ($F=22.70$, $p<0.001$). The dimension averaged 0.58 ± 0.11 in the plants examined. From March to July its range was between 0.52 and 0.58, but significantly increased to 0.66 in October and then decreased to 0.48 in February. The constriction of branches, however, showed no difference among different life history phases (Fig. 5).

Axis diameter significantly varied in different months throughout the year ($F=78.86$, $p<0.001$) and also with different life history phases ($F=7.56$, $p<0.001$). The mean was 0.8 mm in vegetative plants, 0.9-1.0 mm in males and females, and 1.1 mm in tetrasporic ones (Fig. 5). Axis diameter averaged 0.9 ± 0.2 mm during the entire study period. It increased to 1.3 mm in June and then decreased to 0.7 mm in November (Fig. 4).

Medullary cell size significantly varied throughout the year ($F=48.31$, $p<0.001$) and also in different life history phases ($F=42.67$, $p<0.001$). The monthly mean was a minimum of 131.3 ± 27.5 μm in November and a maximum of 250.2 ± 27.4 μm in May (Fig. 4).

Correlation and standardization of features examined: Plant length was positively correlated with both axis diameter ($r=0.55$, $p<0.001$) and medullary cell size ($r=0.39$, $p<0.001$). Axis diameter also showed a very closed correlation with medullary cell size

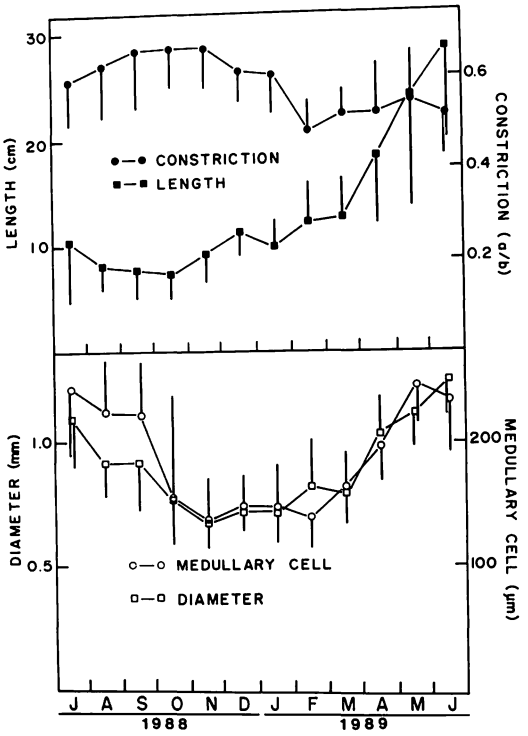

Fig. 4. Monthly variations in morphological features examined in Korean *Gracilaria verrucosa* plants (+ or −SD).

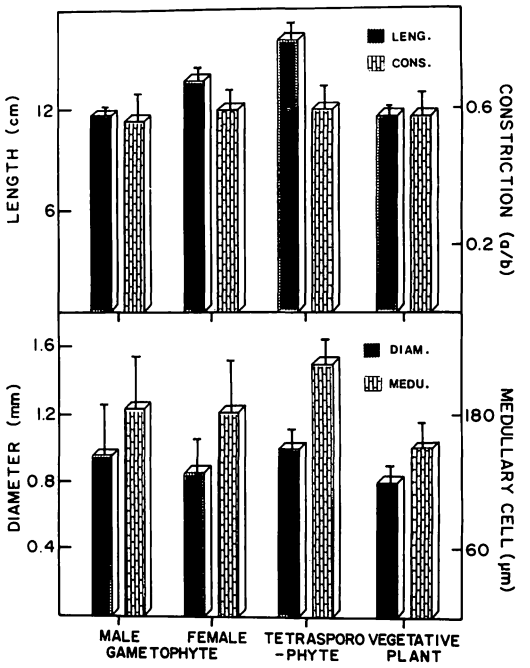

Fig. 5. Relationship between morphological features and life history phases in Korean *Gracilaria verrucosa* plants (+SD)

(r=0.81, p<0.001), whereas plant length showed a weak correlation with medullary cell size (r=0.39, p<0.001). Basal branch constriction was, however, negatively correlated with plant length, axis diameter and medullary cell size. This negative correlation can be visualized: in Figure 6, standardizing values show how the constriction variable contrasts with those of plant length, axis diameter and medullary cell size, while still varying through the months.

Discussion: This result agrees with the previous field and culture studies (Jones 1959, Ogata *et al.* 1972), that *Gracilaria verrucosa* plants have a *Polysiphonia*-type life history. There is also a marked seasonality in reproduction: a summer peak in reproductive plants while a winter peak in vegetative ones. The number of tetrasporic plants is nearly same with the sexual ones, and the ratio of female and male plants is also 1 : 1 throughout the year. This implys that production and survival of spores in Daechon population appears to be same between life history phases. There have been reports on unequal proportions of phases in Norwegian *G. verrucosa* (Rueness and Tananger 1984) and Canadian *Gracilaria* species (Bird *et al.* 1977, Bird 1976), that is a well-known phenomenon in red algal species occurring at the extreme northern limits of distribution (Dixon 1965).

According to Kain (1986), there appears to be a threshold size above which three red algal taxa from England become reproductive due to energy allocation to reproduction than to growth during a certain time of life history. There has been little information on the relationship between size class and reproduction in the genus *Gracilaria*. Korean *G. verrucosa* plants were the most variable in size in June, but all of them had reproductive organs. This requires further intensive study in comparison with other populations.

Although there is a risk of failing to realize that a continuum of morphologies may exist, thallus architecture has been used for key and delimiting red algal species. Of the morpho-

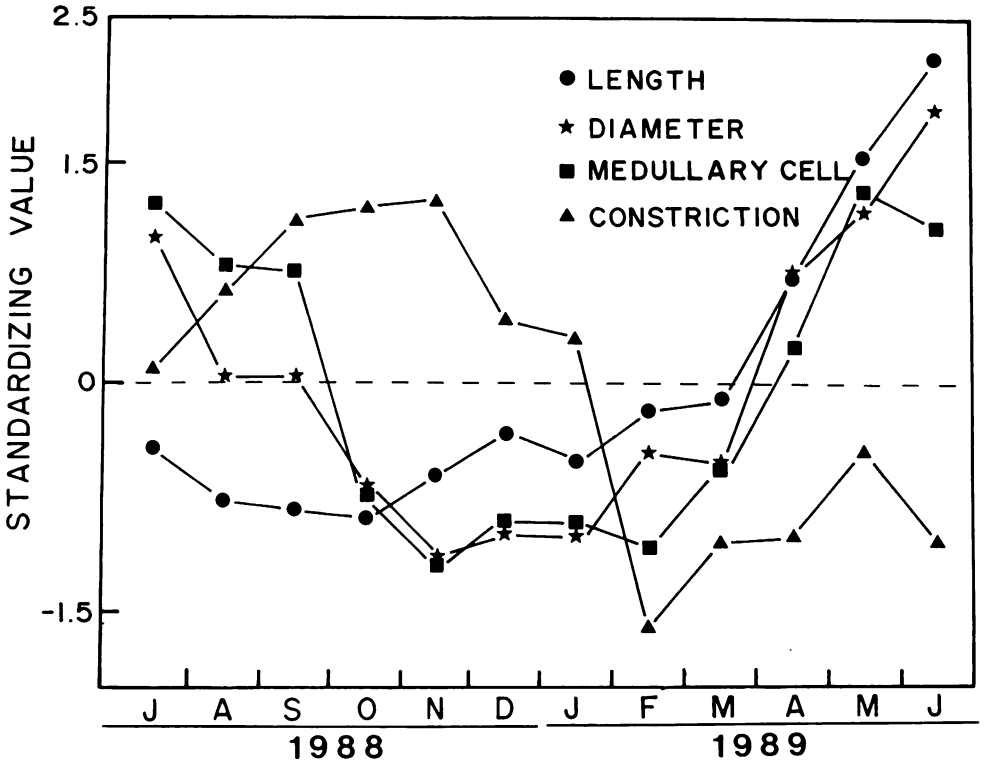

Fig. 6. Monthly variations in four morphological features standardized in Korean *Gracilaria verrucosa* plants.

logical features examined in this study, basal branch constriction has commonly been used as a diagnostic character for the terete *Gracilaria* species (Yamamoto 1984). The dimensions of Canadian plants are very variable in different months and locations (reading from Figures 3 and 4, Chapman *et al.* 1977). In this study, they varied significantly from 0.48 in February to 0.66 in October, without any difference among different life history phases. Hence, branch constriction should be used as a good delimiting feature in mature plants.

Axis diameter ranges in 1-3 mm in Chinese (Zhang and Xia 1985) and Japanese plants (Yamamoto 1978). The monthly mean in Korean plants varied significantly from a minimum of 0.7 mm in November to a maximum of 1.3 mm in June as well as in different life history phases. Plant length and medullary cell size also varied in different months and life history phases, in accordance with Chapman *et al.* (1977). However, correlation coefficients and standardizations of quantified features showed that basal branch constriction was an independent feature, whereas plant length, axis diameter and medullary cell size were dependent.

As growth pattern varies with environmental factors, which in turn influence the phenology of *Gracilaria* species (Bird and McLachlan 1986), different environments may be expected to produce different morphologies. Korean *G. verrucosa* plants show a wide variation in gross morphology, as mentioned in plants from other regions (Chapman *et al.* 1977). The morphological variation may be due to observing features that are both unstable and ill-defined brought about by juvenile plants and by the simplicity of plant architecture.

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Myung Sook Kim*, In Kyu Lee* and Sung Min Boo：韓国西岸における *Gracilaria verrucosa*（紅藻）の季節的消長と形態について**

韓国西岸において *Gracilaria verrucosa* は生殖器官の形成時期において顕著な季節性を示した。成熟藻体は主として夏に優占し，栄養的な藻体は冬に優占した。形態学的特徴は生活史における段階と季節により著しい変異を示した。藻体の高さと直径，髄層の細胞の大きさには統計上有意の相関がみられたが，下部の技にみられるくびれはその他の形態学的形質と相関を示さなかった。(*Department of Biology, Seoul National University, Seoul 151–742, Korea, **Department of Biology, Chungnam National University, Daejon, 305–764, Korea)

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